REMARKS

FORMAL MATTERS:

Claims 1-5, 10, 14-20, 22, 27-28, 30-35, and 40 are pending after entry of the amendments set forth herein.

Claims 6-9, 11-13, 21, 23-26, 29, 36-39, 41 are canceled.

Claims 1 and 15 are amended to recite the endogenous C5aR coding sequences are disrupted. Support is found in the specification at, for example, page 5 lines 20-21; page 7, lines -12; page 28, lines 8-14.

Claims 10 and 27 are amended in view of the amendments to claims 1 and 15

Claims 2, 3, 17, 18 and 28 are amended for further clarity. The amendments to claims 2, 3, 17 and 18 reflect amendment suggested by the Examiners during the telephonic interview discussed below. Claim 28 is amended to include the language found in claim 31 to recite that administering of the candidate compound is "under conditions in which at least one phenotype associated with C5aR signaling is expressed".

These amendments raise no new issues or require a new search, and thus their entry is respectfully requested.

No new matter is added.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

INTERVIEW SUMMARY

Applicants are grateful to Examiner Wilson and Examiner Paras for the telephonic interview conducted on January 5, 2011 with the undersigned.

All rejections of record were discussed during the interview. These rejections include the rejections of the claims under §112, ¶1 for lack of an enabling disclosure and under §103(a) as being unpatentable over the combined disclosures of the cited references. The main points of discussion were generally as follows:

- §112, ¶1 Enablement Rejection:
    - Whether the "cre-lox" construct in Figure 1 was "essential" to making the claimed transgenic mice

- Whether the specification adequately described steps and controls for carrying out the screening method of claims 28, 30-35 and 40

- §103(a) Obviousness Rejection:
  - Whether the result that the endogenous mouse C5a ligand binds human or humanized C5aR can be considered an unexpected result in view of the disclosure of the Cain reference.

As discussed during the interview,

- The use of the cre-lox system in the targeting construct is not "essential" to making the claimed transgenic mice. As discussed below, the C5aR is not essential to development, as supported by reports in the literature of C5aR double knock-out mice. The transgenic mice of the claims can be produced using any suitable targeting construct system.
- Method steps and controls for carrying out the screening method of claims 28, 30-35 and 40 are adequately described in the specification and, in fact, are specifically exemplified in the examples showing that the effects of an anti-C5aR antibody (referred to as "7F3") in inhibiting development of arthritis can be detected in a transgenic human C5aR mouse, with control mice expressing endogenous C5aR serving as a control to show the effect is due to interaction of the agent with the human C5aR transgene product in the transgenic mice.
- The Cain reference does not undermine the unexpected nature of the result that endogenous mouse C5a binds a human or humanized C5aR. Cain is, in fact, irrelevant to this issue since Cain describes making modifications to a *human* C5aR to render the receptor more "mouse-like". Furthermore, Cain does not examine binding of C5a to this modified human C5aR, but rather examines binding of *synthetic drugs.*

The arguments presented below encompass the arguments presented during the interview.

In addition, the Examiners suggested the claims be amended for further clarity to make more clear which sequence identifiers referred to amino acid sequence and which referred to nucleotide sequences, and to recite "the" sequence of the recited sequence identifier. These claims amendments are set forth above.

REJECTIONS UNDER §101

Applicants acknowledge the withdrawal of the rejection of the claims under §101.

REJECTIONS UNDER §112, ¶1 – NEW MATTER

Claims 28, 30-35 and 40 were rejected on the grounds that the phrase "or tissue or cells obtained therefrom" in claim 28 is new matter. The Office Action states that no support was provided and none can be found. This rejection is respectfully traversed.

Support for isolated tissue or cells obtained from a transgenic mouse of the claimed invention can be found at, for example, page 6, lines 8-10; and at page 7, line 32 to page 8, line 2. At page 7, line2 to page 8, line 2, the specification states:

> Accordingly, the present invention also provides a method for evaluating at least one pharmacokinetic and/or pharmacodynamic effect of a candidate compound, the method comprising administering a candidate compound to a transgenic mammal of the present invention or isolated tissue or cells obtained therefrom and examining at least one pharmacokinetic and/or pharmacodynamic effect of the candidate compound on the transgenic mammal.

Notably, as set out in the specification at page 9, lines 23-24, a pharmacodynamic effect encompasses modulation of C5aR signaling activity, which can be observed in isolated tissue or cells obtained from the transgenic mouse.

Withdrawal of this rejection is respectfully requested.

REJECTION UNDER §112, ¶1

Claims 1-5, 10, 14-20, 22, 27, 28, 30-35 and 40 were rejected under §112, ¶1 on the grounds that the claims fail to comply with the enablement requirement. This rejection is respectfully traversed as applied and as it may be applied to the presently pending claims.

Technical review

The present invention provides a mouse that is transgenic for a human or humanized C5a receptor ("C5aR").

C5a is a proteolytic product of a larger complement protein (see the specification at page 1, lines 13-33). Proteolysis of complement proteins occurs in response to inflammation or the formation of antigen/antibody complexes (immune complexes). Thus production of C5a occurs *after* an inflammatory

response is induced or as a result of complement activation by immune complexes. Once produced, the C5a ligand binds the C5aR, which in turn mediates the development of conditions that can result from such inflammatory responses, such as arthritis. As explained in the specification at page 40, line 28-30, C5aR knock-out mice do not develop inflammation in the KRNxNOD (K/BxN) serum transfer model of rheumatoid arthritis.

In order to induce C5a production, an agent that triggers an inflammatory response that results in production of C5a from complement proteins must be administered to the mouse. As discussed in the specification at page 60, lines 5-8, sera from arthritic K/BxN mice (referred to here as "arthritis-inducing sera") induces an inflammatory response which leads to development of arthritis in mice. Notably, this arthritis-inducing sera induces arthritis in *healthy animals.*

Because the transgenic human C5aR mice have the endogenous mouse complement proteins, administration of arthritis-inducing sera results in production of mouse C5a. The transgenic human C5aR mouse exemplified in the present specification is a homozygous knock-in for the human C5aR, and thus does not express the endogenous mouse C5aR. Thus any C5aR-mediated effect is due to *binding of endogenous mouse C5a to the human or humanized C5aR.* This is not to suggest that mice transgenic for a human or humanized C5aR, and that express one or both of the endogenous mouse C5aRs, would not work. Rather, the use of a homozygous knock-out in these experiments avoids the possibility that the effects observed were due to mouse C5a binding to an endogenous mouse C5aR.

Evidence that development of arthritis in the transgenic human C5aR mouse was due to the mouse C5a binding the human C5aR transgene product is set out in the specification page 62, line 20 to page 63 line 4). Arthritis-inducing sera was administered to transgenic human C5aR mice and to wild-type/control mice. The transgenic human C5aR mice and the wild-type control mice then received an anti-human C5aR antibody (referred to as 7F3) or a control antibody of irrelevant specificity. The anti-human C5aR antibody does not bind mouse C5aR.

The anti-human C5aR antibody inhibited development of clinical signs of inflammation *only in the transgenic human C5aR mice*. The wild-type/control mice that received the anti-human C5aR antibody still developed arthritis symptoms, confirming that the arthritis symptoms observed in the transgenic mice were due to C5a binding to human C5aR. The control antibody did not inhibit development of arthritis symptoms in either the transgenic mice or the wild-type/control mice, showing that the inhibition of arthritis by the anti-human C5aR antibody in the transgenic mice was not an

artifact of antibody administration. Thus, blocking of endogenous mouse C5a binding to the human C5aR in the transgenic mice inhibited development of arthritis.

In summary, the specification shows that arthritis in the transgenic human C5aR mice is due to the binding of the endogenous mouse C5a ligand to the human C5aR:

- Mice transgenic for human C5aR, but which have endogenous mouse C5a ligand, develop arthritis following administration of arthritis-inducing sera *just as do nontransgenic control mice*; and
- An antibody specific for human C5aR blocks induction of arthritis in transgenic human C5aR mice, *but not in nontransgenic control mice*.

These data show that endogenous mouse C5a binding to human C5aR in the transgenic human C5aR mice mediates development of arthritis following induction of inflammation by arthritis-inducing sera.

Applicants now turn to the rejection at hand.

Overview of the rejection under §112, ¶1

The primary grounds in support of the rejection under §112, ¶1 are as follows:

1. The targeting construct used to make the transgenic mice is not adequately described in that a) the region of the "mouse-human fusion", b) the area of the mouse C5aR replaced with human sequences, and c) the promoter driving expression of the human C5aR sequences are unclear;
2. The targeting construct uses a cre-lox system, which the Examiner asserts may be essential;
3. The methods of screening (claim 28 and dependents) are not enabled as the method steps and controls are not adequately described; and
4. The specification fails to describe how to use a mouse expressing both human C5aR while still expressing their endogenous C5aR gene.

Each of these grounds in support of the enablement rejection are addressed below.

1) The targeting construct and the structure of the transgene are adequately described

The Office Action at page 5 states:

> Accordingly, the specification fails to adequately teach how to make the mouse of claim 1 or use the method of claim 15. Pg 51, lines 9-16, discusses Fig. 1, which describes the targeting construct used to make the transgenic mice in the Examples. However, the structure of the targeting construct is not readily apparent from Fig. 1. In particular, the region of "mouse-human fusion" is unclear and does not teach what area of the mouse C5aR has been replaced with human sequences or what promoter is driving the human C5aR sequences.

At page 8, in response to the arguments presented earlier, the Office Action states:

> The sequence of the targeting locus, i.e. the C5aR gene, was known in the art at the time of filing and is not the issue. The issue is that the structure of the targeting construct made by applicants, i.e. the elements that went into the construct and their order, is not disclosed.

This rejection is respectfully traversed.

The specification describes the construct in detail, including the details of the sequences provided in the targeting construct (see, e.g., specification page 52, lines 11 to 35).

With specific reference to the Examiner's request regarding a) the "mouse-human fusion", b) the area of the mouse C5aR replaced with human sequences, and c) the promoter driving human C5aR expression, the specification states:

> The targeting vector used to generate the knock-in mice includes regions homologous to approximately 3kb genomic DNA either side of exon 3 (i.e. from about nucleotides 7377-15045 as shown in SEQ ID NO:1). In particular, the targeting vector comprised the region from about nucleotides 7377-15045 of SEQ ID NO:1 except that nucleotides 10726-11778 were replaced by nucleotides 28 to 1077 of SEQ ID NO:2. This means that following integration, the endogenous mouse exons 1 and 2 remain in the transgenic mammal but exon 3 of the mouse locus has been replaced with a sequence encoding human C5aR.

(specification page 52, lines 28-35, emphasis added).

In view of the above, a skilled person would understand that the control sequences driving expression of the humanized C5aR in the transgenic mouse are the native mouse sequences.

The region of the mouse-human fusion in the targeting vector, which is described precisely in the specification as shown above, determines the site of integration of the construct into the mouse genome and the sequence of the resultant humanized C5aR sequence that is left in the mouse genome after targeted integration has occurred. A skilled person would understand that the other components of the vector, such as marker genes and cre-lox elements, and the arrangement of those components in the vector, are not essential to the working of the invention and could be substituted with functionally similar components or deleted from the vector.

Withdrawal of this rejection is respectfully requested.

The Office Action at page 5 also states "Applicants' arguments regarding obviousness include mention of unexpected results that human C5aR would bind mouse C5a. Assuming that is true, this goes toward unpredictability of the invention …". Applicants' respectfully traverse.

The enablement rejection is directed toward how to make and use a mouse expressing a human or humanized C5aR transgene. The unexpected results presented in response to the obviousness rejections relate to *whether the ordinarily skilled artisan would expect the endogenous mouse C5a ligand to bind the human or humanized C5aR transgene product*. Applicants have shown in the specification the successful production of an example of a transgenic human or humanized C5aR mouse and also shows that this mouse develops arthritis that is mediated by binding of the endogenous mouse C5aR to the human C5aR transgene product.

Withdrawal of this rejection is respectfully requested.

2) A cre-lox system is not required to make the claimed transgenic mice

The Office Action page 5 states:

> It is also noted that the targeting vector requires a cre-lox system which is not in the claims. If cre-lox elements are essential in the targeting vector to make the mouse, then the structure of the targeting vector must be included in the mouse claimed and include the cre-lox elements that are essential to make the mouse.

In response to Applicants' arguments submitted in the Amendment filed July 1, 2010 (referred to as the "July 2010 Amendment"), the Office Action states at pages 8-9:

> The Cre-Lox system is employed to prevent embryonic lethality; therefore, the structure of the Cre-Lox system used by applicants is essential to prevent embryonic lethality. Use of selectable markers has nothing to do with preventing embryonic lethality.

The Examiner clarified during the interview that this grounds for the enablement rejection is based on the concern the C5aR may be essential to development, and thus the cre-lox system must be used in order to generate viable transgenic mice. Applicants respectfully traverse.

The C5aR gene is not essential to development. In support, Applicants submit with this response Hopken et al. (1996) Nature 383:86-89 ("Hopken").[1] Hopken describes production of mice deficient in the endogenous C5aR . Hopken at page 86 discloses that these mice expressed no detectable C5aR:

[1] Applicants respectfully request the Examiner enter and consider this reference as evidence despite the status of the present application as being after final. Until the explanation provided in the Final Office Action, it was not apparent that this ground of rejection was based on the concern that the C5aR gene was essential to development.

> The murine C5a receptor (C5aR) gene[4] was deleted through homologous recombination with the targeting vector shown in Fig. 1*a*. Correctly targeted J1 embryonic stem cells were used to generate chimaeric mice, several of which transmitted the targeted allele through the germ line (Fig. 1*b*). To confirm that C5aR was not present in the targeted animals, binding and signalling to exogenous recombinant mouse C5a and northern blot analyses were performed (Fig. 1*c*, *d*). Northern analysis of bone-marrow messenger RNA from rC5aR-deficient ($C5aR^{-/-}$) mice demonstrated that there was no hybridization with the C5aR complementary DNA coding sequence probe (Fig. 1*d*).

Hopken thus provides evidence that a mouse deficient in C5aR (i.e., a double C5aR knockout) is viable The C5aR gene is thus not essential to development. Production of mice lacking one or both endogenous C5aR genes is thus enabled by the present specification when combined with the knowledge of the ordinarily skilled artisan at the time of the priority date of the present claimed invention.

Applicants note that, as set out in the specification at page 51, lines 31-35, the rationale for using the cre-lox system is to allow for removal of the selectable marker.

Withdrawal of this rejection is respectfully requested.

3) The specification provides adequate guidance to enable claims directed to methods of screening

The Office Action at page 6 states:

> Claim 28 is drawn to evaluating a compound by administering the compound to the mouse of claim 1 (or isolated tissues or cells obtained therefrom) and examining a pharmacokinetic/pharmacodynamic effect of the compound. However, the specification fails to enable those of skill to determine how to use the mouse of claim 1 to screen drugs.

The Office Action also states at page 7:

> Overall, it is unclear how the "homozygous hC5aR and wild-type (control) mice" are "in the K/BxN model" as described by applicants. Second, it was predetermined that the anti-human C5aR antibody targeted hC5aR and not mouse C5aR, so the controls required to identify compounds that specifically target hC5aR using the mice claimed are not described by applicants. Pg 62, lines 20-35, discuss a method of screening drugs using homozygous human C5aR knockin mice without teaching the specific steps required to do so. Applicants have left those skilled in the art with no information how to use the non-human mammals claimed to identify compounds that target human C5aR. Finally, merely observing whether a compound known to specifically target human C5aR decreases inflammation in a knockin mouse (given K/BxN sera?) as compared to a control is not an enabled use in and of itself because the compound was already known to treat disease.

In response to the arguments presented in the July 2010 Amendment, the Office Action states at page 9:

> Applicants argue claim 28 is enabled because pg 60, lines 11-15, teach sera from arthritic K/BxN mice was injected into homozygous C5aR and wild-type control mice. Applicants' argument is not persuasive for reasons set forth above.
>
> ***
>
> Claim 28 is not limited to using a mouse given K/BxN serum. Furthermore, applicants have not explained how to perform the method and identify agents that target C5aR, i.e. what controls are required to identify agents that target C5aR? Merely looking for inflammation in the mouse does not necessarily indicate the agent is acting on the C5aR.

Applicants respectfully traverse.

It is true that merely looking for inflammation in a mouse does not *necessarily* mean that the agent is acting on C5aR. However, this does not detract from the usefulness of the transgenic mouse of the invention. As explained in the specification, the transgenic mouse of the invention is useful for performing an important assessment of therapeutic agents that have already been identified as targeting C5aR in *in vitro* studies. The specification provides the following relevant background at page 3, lines 1 to 12:

"A critical requirement in the drug discovery process is the demonstration in relevant animal models that new therapeutic agents identified by *in vitro* screening methods are safe and effective. Moreover, it is often desirable to compare the *in vivo* efficacy of numerous agents to select for desirable properties including pharmacokinetic properties, efficacy in affecting disease outcome and lack of adverse side effects. One of the main obstacles to drug development is moving drug candidates from *in vitro* assays to demonstration of in vivo efficacy. Often this is because many drug candidates are species-specific. For instance, an antagonist developed to a human chemoattractant receptor, such as C5aR, might antagonise only human C5aR and not C5aR from mouse, rabbit or even higher primates. The inability of many drugs candidates to "work" across species is a major reason for attrition in the preclinical phase."

At page 3, lines 20 to 25 the specification states:

"The present inventors have found that a number of C5aR antagonists react with human C5aR but not C5aR from other species. For example, monoclonal antibodies MAb 7F3, MAb 6C12 and MAb 12D4 (described in PCT/AU03/00084) bind to human C5aR but do not bind to mouse or baboon C5aR. This brings to light the need for an *in vivo* screening and validation system that is capable of detecting and/or validating agonists/antagonists that are specific for human C5aR."

In light of these passages a skilled person would understand that the transgenic mouse of the invention can be used in the evaluation or validation of candidate drugs targeting C5aR that have already been identified in *in vitro* tests. In other words, the transgenic mouse of the invention is unlikely to be used in isolation to identify new drugs that target C5aR, rather it would be used for the critical *in vivo* validation or evaluation of agents that have already been identified in preliminary *in vitro* tests as binding to C5aR.

With that background in mind, the method steps and controls for carrying out the screening method claims of claims 28, 30-35 and 40 are adequately described in the specification and, in fact, are specifically exemplified. The anti-C5aR antibody (referred to as "7F3") serves as proof of principle that one can use the claimed transgenic mouse to detect the *in vivo* effects of a compound that binds to C5aR.

Specifically, Applicants showed in the specification at page 62, line 20 to page 63 line 4 an example of how such a screening method could be carried out. Arthritis-inducing sera was administered to transgenic human C5aR mice and to wild-type/control mice to create a condition under which a phenotype associated with C5aR signaling is expressed. The wild-type/control mice were C57BL/6

mice, and thus expressed only endogenous mouse complement proteins (and thus have endogenous mouse C5a) and endogenous mouse C5aR (specification page 61, lines 16-18).

The transgenic human C5aR mice and the wild-type control mice then received a candidate compound –an anti-human C5aR antibody (referred to as 7F3) or a negative control antibody (an antibody of irrelevant specificity – i.e. which does not bind to C5aR). The anti-human C5aR antibody inhibited development of clinical signs of inflammation *only in the transgenic human C5aR mice*. The antibody of irrelevant specificity did not inhibit development of arthritis symptoms in either the transgenic mice or the wild-type/control mice.

In summary, this example shows how mice transgenic for human C5aR can be used to evaluate a candidate agent which binds to C5aR for *in vivo* efficacy.

Withdrawal of this rejection is respectfully requested.

4) The specification provides adequate guidance to use transgenic mice that express a human or humanized C5aR and still express their endogenous C5aR

The Office Action at pages 7- 8 states:

> Claims 1-5, 10, 12, 14-20, 22, 27, 28, 30-35 and 40 encompass mice expressing human or humanized C5aR while still expressing their endogenous C5aR gene. The specification and the art at the time of filing do not teach how to use a mouse expressing both human C5aR while still expressing their endogenous C5aR gene. The specification is limited to a transgenic mouse whose genome comprises a homozygous disruption in a mouse C5a receptor gene, and whose genome is homozygous for a nucleic acid sequence encoding human C5aR. Thus, it would have required those of skill in the art at the time of filing undue experimentation to determine how to use heterozygous C5aR mice to screen compounds, and the claims should be limited to homozygous C5aR mice.

Without conceding to this ground of rejection, claims 1 and 15 are amended to recite that the endogenous C5aR genomic sequences are disrupted. Withdrawal of this rejection is respectfully requested.

REJECTIONS UNDER §103(A)

The Examiner has maintained the rejection of claims 1-5, 10, 14-20, 22, 27, 28, 30-35 and 40 as allegedly being obvious over Sato (Thrombosis and Haemostasis (1999) 82(2):865-869), Roebroek (Methods in Molecular Biology (2003) 209:187-200), Homanics (Methods in Alcohol Related Neuroscience Research (2002), pg. 31-61), Lester et al, (Curr. Opin. Drug Discov. and Dev. (2003) 6(5):663-639), Champtiaux (Curr. Drug Targets, CNS & Neuro. Dis.(2002)1:319-330), Girardi (J. Clin. Invest. (2003) 112(11):1644-1654) in view of Burmer (WO 02/61087) and Cain (Biochemical Pharm., 2001 61:1571-1579) and as supported by Drago (Cell. Mol. Life Sci 2003 60:1267-1280), Gu (Dev. Cell 2003 5:45-57), Belmont (WO 2002/059262) and Kane (WO 2003/027252).

This rejection is respectfully traversed as applied and as it may be applied to the claims as now pending.

During the interview, the Examiner asked that in response, Applicants step through each reference above, noting the purpose for which the reference was cited. Accordingly, Applicants note the following:

Sato is cited for its alleged disclosure of a knock-in mouse in which an endogenous gene is replaced with an exogenous gene or a mutant form of the endogenous gene.

Roebroek is cited for its alleged disclosure of methods for making knock-in mice, including disrupting an endogenous mouse gene and replacing it with the human homolog.

Homanics is cited for its alleged disclosure of a transgenic mouse having an endogenous receptor disrupted and replaced with a human receptor homolog.

Lester and **Champtiaux** are cited for their alleged disclosure of receptor knock-in mice.

Girardi is cited for its alleged disclosure of "knocking out" a mouse C5aR gene.

Burmer is cited for its alleged disclosure of human C5aR cDNA.

Cain is cited for its alleged disclosure of mutated human C5aR that functioned in rat cells.

Drago, **Gu**, **Belmont** and **Kane** are cited for their alleged disclosure of a humanized receptor that bound a mouse ligand.

Applicants' response as to the merits of the rejection is provided below.

Turning to the Office Action's remarks in response to the arguments presented in the July 2010 Amendment, the Office Action at page 12 states:

> Applicants argue those of skill would not expect the human C5aR in the transgenic claimed to be activated by mouse C5a or that it would function. Applicants point to the Declaration by Dr. Gerard which shows the homology of the extracellular domain of mouse, rat and human C5aR. Applicants' arguments and the declaration are not persuasive.
>
> First, the specification and applicants' arguments fail to show mouse C5a "binds to and effect signaling of the human C5aR" in the transgenic mouse as claimed.

The Office Action at page 12 further states:

> Next, the transgenic mice described by applicants did not have a normal phenotype; therefore, it is unclear mouse C5a does "bind to and effect signaling of the human C5aR".

Applicants respectfully traverse.

As explained during the interview and set out above, as well in the specification at page 1, lines 13-33, C5a is a proteolytic product of a larger complement protein. Proteolysis of complement proteins occurs following induction of an inflammatory response. Thus production of C5a occurs *after* an inflammatory response is induced. Once produced, the C5a ligand binds the C5aR, which in turn mediates the development of conditions that result from such inflammatory responses, such as arthritis.

In the mouse model, an inflammatory response that leads to development of arthritis is triggered by administration of sera from arthritic K/BxN mice (referred to here as "arthritis-inducing sera"). As noted in the specification at page 60, lines 5-8, this sera induces arthritis in *healthy animals*.

The specification provides ample evidence that the mouse C5a ligands binds to the human C5aR in the transgenic mice.

The claimed transgenic mice express complement proteins endogenous to the mouse. Development of arthritis symptoms is mediated by binding of C5a to C5aR. Importantly, as explained in the specification at page 40, line 28-30, C5aR knock-out mice do not develop inflammation in the KRNxNOD (K/BxN) serum transfer model of rheumatoid arthritis. Thus, a functional C5aR is required for the development of arthritis symptoms.

Arthritis-inducing sera induced arthritis in both homozygous human C5aR knock-in mice and in wild-type/control mice as evidenced by inflammation, increased ankle thickness, bone erosion and leukocyte infiltration into synovial joints (specification at page 62, lines 1-15). The control mice were wild-type C57BL/6 mice, and thus expressed only endogenous mouse complement proteins (and thus have endogenous mouse C5a) and endogenous mouse C5aR (specification page 61, lines 16-18).

The specification shows that development of arthritis in the transgenic human C5aR mice was due to the mouse C5a binding human C5aR (specification page 62, line 20 to page 63 line 4). Arthritis-inducing sera was administered to transgenic human C5aR mice and to wild-type/control mice. The transgenic human C5aR mice and the wild-type control mice then received an anti-human C5aR antibody (referred to as 7F3) or a control antibody of irrelevant specificity. The anti-human C5aR antibody does not bind mouse C5aR. The anti-human C5aR antibody inhibited development of clinical signs of inflammation *only in the transgenic human C5aR mice*. The wild-type/control mice that received the anti-human C5aR antibody still developed arthritis symptoms, confirming that the arthritis symptoms observed in the transgenic mice were due to C5a binding to human C5aR. The control antibody did not inhibit development of arthritis symptoms in either the transgenic mice of the wild-type/control mice, showing that the inhibition of arthritis by the anti-human C5aR in the transgenic mice was not an artifact of antibody administration. Thus, blocking of endogenous mouse C5a binding to the human C5aR in the transgenic mice inhibited development of arthritis.

In summary, the specification shows that arthritis in the transgenic human C5aR mice is due to the binding of the endogenous mouse C5a ligand to the human C5aR:

- Mice transgenic for human C5aR, but which have endogenous mouse C5a ligand, develop arthritis following administration of arthritis-inducing sera *just as do nontransgenic control mice*; and
- An antibody specific for human C5aR blocks induction of arthritis in transgenic human C5aR mice, *but not in nontransgenic control mice*.

These data show that endogenous mouse C5a binding to human C5aR in the transgenic human C5aR mice mediates development of arthritis following induction of inflammation by arthritis-inducing sera.

Withdrawal of this rejection is respectfully requested.

The Cain reference

With respect to the Cain reference, the Office Action at pages 12-13 states:

> Next, Cain taught mutated human C5aR that were made to "resemble mouse at these positions" functioned in rat cells (pg 1573, col. 2, section 3.2). Therefore, despite the lack of 100% homology of mouse and human C5aR, those of ordinary skill would have known how to make a mutated human C5aR that functioned in murine cells and had a reasonable expectation of mouse C5a binding human C5aR as evidenced by Cain.
>
> It is noted that the claims encompass using any "humanized C5aR" which encompasses any mutation that makes the mouse C5aR more like human C5aR (including the mutation described by Cain or any single amino acid substitution that make the mouse C5aR more like human C5aR). The claims are not limited to replacing the entire mouse gene with the entire human gene.
>
> It is also noted that applicants' arguments regarding Cain indicate a difference in binding of known agonists to mouse and human C5aR; however, variation in binding of agonists to mouse and human C5aR fails to indicate mouse C5a will not bind and effect signaling of human C5aR.

Applicants respectfully traverse.

As discussed during the interview, Cain is simply not relevant to the present claims. The authors of Cain are investigating why the synthetic C5aR antagonist, phenylalanine [L-ornithine-proline-D-cyclohexylalanine-tryptophan-arginine] (referred to as "F-[OPchaWR]") has about 1,000-fold less affinity for mouse C5aR than for the human C5aR (see abstract). After pointing out that the mouse receptor contains a Y at a position analogous to P^{103} in the human receptor and D at human receptor position G^{105}, Cain produced *mutants of the human receptor that contain the mouse receptor amino acid residues* to examine the effect upon *binding to a synthetic drug.* Rather than "humanize" a mouse C5aR, Cain teaches "mouse-cizing" a human C5aR. Furthermore, Cain does not examine binding of a natural C5a ligand, but instead examines binding of *synthetic ligands*.

Cain thus contains no disclosure relevant to the claimed invention, and provides no guidance to the ordinarily skilled art as to whether naturally-occurring mouse C5a ligand would bind a human or humanized C5aR as required by the present claims.

In short, Cain discloses modification of a *human receptor*, not modification of a non-human receptor to contain amino acid sequences of a human receptor.

Withdrawal of this rejection is respectfully requested.

The Drago reference

With respect to Drago, the Office Action states at page 12:

> Applicants argue Drago did not teach humanized receptor bound mouse ligand. Applicants' argument is not persuasive. Pg 1274, col. 1, 2nd partial paragraph, shows a leucine-to-serine point mutation in a critical residue within the second transmembrane domain of the α4 nAChR subunit (L9'S knockin) effects signaling which implies binding of ligand to the receptor.

The passage of Drago to which the Office Action appears to refer is as follows:

> The interaction between the α_4 subunit and dopaminergic systems has also been explored by other investigators, using transgenic mice with a leucine-to-serine point mutation in a critical residue within the second transmembrane domain of the α_4 nAChR subunit (L9`S knockin). This mutation results in increased sensitivity of $\alpha_4\beta_2$ receptors to agonists. Even in the hemizygous state the knockin mutation results in dramatic late embryonic loss of midbrain dopaminergic neurons [89]. These mutants fail to feed and die in the first postnatal day. The cell death was possibly due to the persistent activation of nAChRs by circulating choline, which in low concentrations was shown to be an agonist at mutant $\alpha_4\beta_2$ nAChRs but not at normal $\alpha_4\beta_2$ receptors.

(Drago, page 1274, col. 1, par. 2 (partial).

Applicants respectfully traverse.

As discussed during the interview, "humanized C5aR" refers to a non-human C5aR that is modified to have a human C5aR sequence. Drago fails to teach or suggest modification to a mouse

nAChR to introduce a characteristic of a human nAChR.[2] There is nothing in Drago that teaches or suggests that the modification to which the Office Action points – a leucine-to-serine point mutation in the mouse nAChR – is a change that introduces a human amino acid sequence.

In this passage cited in the Office Action, Drago points to work by Labarca (2001) Proc. Natl. Acad. Sci. 98:2786-279 (cited as reference 89 in Drago; copy enclosed).[3] Labarca also fails to teach or suggest "humanizing" the mouse nAChR. Labarca only discloses that a single point mutation in the mouse nAChR resulted in a hypersensitive mouse nAChR. There is no teaching or suggestion to modify the mouse nAChR with a human amino acid sequence. An alignment of the mouse nAChR with a human nAChR reveals that, in fact, there is NO position at which the mouse sequence contains a leucine and the human sequence contains a serine.

Withdrawal of this rejection is respectfully requested.

The Gu reference

With respect to the Gu reference, the Office Action states at page 12:

> Applicants argue Gu did not teach humanized receptor bound any of the multiple possible mouse ligands. Applicants' argument is not persuasive. Gu taught the humanized receptor effected signaling which implies binding of at least one of the multiple ligands to the receptor.

Applicants respectfully traverse.

The Gu reference describes a study of Neuropilin-1 (Npn-1) receptor which binds multiple ligands from structurally distinct families such as semaphorins (Sema) and vascular endothelial growth factors (VEGF). Applicants respectfully submit that their close review of the disclosure of Gu failed to reveal any disclosure of a humanized receptor. Indeed, Gu points to a *bovine* sequence for guidance in making the amino acid changes to the mouser Npn-1 receptor.

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[2] This analysis is supported by the analysis provided by Dr. Craig Gerard in his §1.132 Declaration submitted with the July 2010 Amendment (see Declaration of Craig Gerard, MD, PH.D. Under 37 C.F.R. §1.132, par. 21, filed July 1, 2010 ("Gerard Declaration")).

[3] Applicants respectfully request the Examiner enter and consider this reference as evidence despite the status of the present application as being after final. Until the explanation provided in the Final Office Action, it was not apparent that this ground of rejection was focused on the single amino acid change as providing for a "humanized" receptor.

In describing the transgenic mouse produced, Gu states at page 47, col. 1, first full paragraph:

> To unravel Npn-1's ligand- and cell-type-specific functions and thereby shed light on the regulation of cardiovascular and neuronal development, we sought to selectively disrupt Npn-1 interactions with semaphorins while retaining interactions with VEGFs. Guided by the structure of the bovine Spermadhesin CUB domain (Romero et al., 1997), we identified 7 amino acids located on two adjacent hydrophilic loops of the amino-terminal Npn-1 CUB domain that are critical for binding to the Sema domain of class 3 semaphorins. Substitution of these 7 amino acids completely disrupts Sema-Npn-1 binding but does not affect $VEGF_{165}$-Npn-1 binding or $VEGF_{165}$'s ability to associate with and activate its signaling receptor, VEGFR2 (Gu et al., 2002). Here, we describe a mouse mutant (*npn-1*$^{Sema-}$) that was generated by altering the coding determinants of these 7 amino acids within the *npn-1* locus by homologous recombination in ES cells. The *npn-1*$^{Sema-}$ mouse expresses normal levels of Npn-1 protein, but Sema-Npn-1 signaling is abolished while VEGF-Npn-1 signaling is retained. For complementary analyses, we also used a Cre-loxP strategy to generate a conditional *npn-1* null mouse. Furthermore, we crossed *npn-1*$^{Sema-}$ mice and *npn-2* null mice to generate double mutant mice in which all secreted semaphorin signaling is abolished. Analyses of these mutant mice allow us to determine the ligand- and cell-type specificity of Npn-1 function in vivo. Our findings indicate that Npn-1 coordinates the activities of structurally distinct ligands that control the development of the heart, vasculature, and nervous system.

(emphasis added)

At best, Gu describes making a modified mouse receptor that contains amino acid sequences of a bovine receptor – a "bovinized" receptor, not a "humanized" receptor. There is nothing in Gu that teaches or suggests making a humanized receptor. Moreover, since the modified receptor is a modified *mouse* receptor that is expressed in a transgenic *mouse,* Gu also fails to provide any evidence that a mouse ligand binds a human or humanized receptor.

Withdrawal of this rejection is respectfully requested.

Remaining references

As admitted by the Examiner, none of Sato, Roebroek, Homanics, Lester and Champtiaux, or

Burmer teach a human C5aR knock-in transgenic mouse. Girardi teaches a knock-out C5aR gene in mice and Burmer discloses the human C5aR sequence. The remainder of the references provide background on knock-in technology or knock-ins of genes other than C5aR. There is no suggestion in any of these references, alone or in combination, to make a human C5aR knock in mouse. There was no motivation to make a human C5aR knock in mouse because at the priority date it was not known or predictable:

- Whether mouse C5a, the endogenous ligand, would bind efficiently to exogenous human C5aR *in vivo* and activate human C5aR signaling; or
- Whether mouse C5a would cause chemotaxis of mouse leukocytes expressing human C5aR.

In paragraph 16 of his Declaration, Dr Gerard provides evidence that cross species functions of ligands such as C5a and their respective G protein coupled receptors were unpredictable at the priority date.

The following references provide further prior art examples of mouse ligands that do not bind to the corresponding human receptor:

- Layton *et al.*, 1994, J Biol. Chem. 269(25): 17048-17055 which shows that human LIF can bind mouse LIF-receptor(LIF-R), but that mouse LIF does not bind human LIF-R.

- Mosmann *et al.*, 1987, J. Immunol. 138:1813-1816 which shows that mouse IL-4 does not bind human IL-4R, and that human IL-4 does not bind mouse IL-4R.

- Liu *et al.*, 1996, Cytokine 8(8): 613-621 which shows that human IL-2 does bind both mouse and human IL-2R equally, that mouse IL-2 binds mouse IL-2R, but that mouse IL-2 does not bind human IL-2R very well.

In another prior art example, Smith *et al.*, 1986, J. Biol. Chem. 261(32): 14871-14874, show that

mouse and human TNF bind human TNF-receptor (TNF-R) with similar affinities but that human TNF binds to mouse TNF-R with considerably lower affinity than mouse TNF binds to mouse TNF-R. This relationship is opposite to that observed for LIF and IL-2.

The diversity of cross-species ligand-receptor interactions provided by these examples illustrate that the binding of a ligand of one species to its corresponding receptor in another species cannot be predicted

These references provide further evidence that a skilled person would not have been able to predict whether mouse C5a would bind to human C5aR.

Summary

The combined references fail to teach of suggest the claimed transgenic mouse, and further fail to provide any reasonable expectation that a human or humanized C5aR would bind a mouse C5a.

Withdrawal of the rejection of the claims under §103(a) is respectfully requested.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number RICE-050.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS LLP

Date: February 8, 2011

By: /Carol L. Francis, Reg. No. 36,513/
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Registration No. 36,513

Enclosure(s): Hopken et al. (1996) Nature 383:86-89
Labarca (2001) Proc. Natl. Acad. Sci. 98:2786-279

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